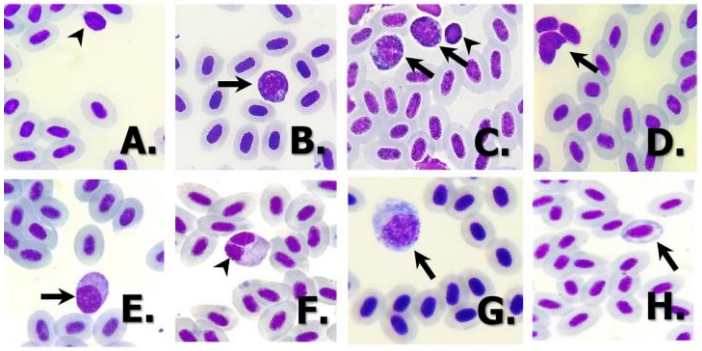
# Silver Nanoparticle-Induced Lymphocyte Activation in Zebrafish

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**Abstract (300-word limit)**

Silver nanoparticles (Ag-NPs) are among the most widely used nanomaterials in the world. Nanotoxicological data on these additives remain unclear. The aim of this study is to evaluate the *in vivo* effects of Ag-NPs bioaccumulation on the hematological cells of adult zebrafish *Danio rerio* after three weeks of oral exposure. Four concentrations of Ag-NPs (65 nm nanosphere) are used: control group 1 (0.00 g/kg of food), group 2 (0.05 g/kg of food), group 3 (5 g/kg of food) and group 4 (50 g/kg of food). The experiment is conducted in triplicate. Cytological and histological results show, a significant increase in neutrophils associated with a significant decrease in lymphocytes in both group 3 and 4 compared to the control group ($p<0,05$ and $p<0,005$, respectively). This reflects an elevated neutrophil/lymphocyte ratio (NLR), suggesting a biased immune response associated with hyperplasia of the hematopoietic tissue and histopathological images of nephrological lesions, following medium to high dose of Ag-NPs intake. Besides, microscopic observation of cell morphology revealed lymphocyte activation only in groups 2 and 3 ($p<0,005$ and $p<0,05$, respectively).
These results suggest that Ag-NPs induce a dose-dependent bioeffects on the immune system in zebrafish. This study reports that at low doses, Ag-NPs may activate lymphocytes but do not alter the NLR, thus demonstrating an immunostimulatory effect. The present investigation is the first study, as far as we know, reporting that Ag-NPs induce a dose-dependent immunomodulation in zebrafish.

**Image**




**Zebrafish blood cells.** Modified-Giemsa-Stained zebrafish blood smear.
(A - C) arrowhead: Lymphocyte core chromatin nucleus with a very high nucleoplasmic ratio. arrow: Activated lymphocyte, increased size with very basophilic cytoplasm.
(D) arrow: Platelet aggregates, notice that platelets are like lymphocyte, however the nucleus is oval, flattened with a very dense chromatin.
(E - F) arrow: Neutrophil with a peripheral nucleus with abundant cytoplasm. arrowhead: Neutrophil with segmented nucleus.
(G) arrow: Monocyte with large cell, abundant gray-sky cytoplasm, and several vacuoles.
(H) arrow: Polychromatophil, notice the large cell with a heterogeneous basophilic cytoplasm compared to mature red blood cells.
Blood smear images were photographed under 1000 x magnification with immersion oil.

**Recent Publications (minimum 5)**

1. Mahjoub T, Chernine S, Lahmar R, Gourain V, Mahjoub MS, Rejeb A, Romdane MN, Ben Romdhane S, Abdelmelek H (2022) Silver Nanoparticle-Induced Lymphocyte Activation in Zebrafish. Nanomedicine Research Journal, 7(2): 195-204. doi: 10.22034/nmrj.2022.02.009
2. Akaichi A, Jebali A, Benlarbi M, Mahjoub T, Kaboudi K, Chaouacha-Chekir RB, ... & Boudhrioua N (2022) Effects of humic acid and organic acids supplements on performance, meat quality, leukocyte count, and histopathological changes in spleen and liver of broiler chickens. Research in veterinary science, 150, 179-188. https://doi.org/10.1016/j.rvsc.2022.07.001
3. Mahjoub T, Krafft E, Garnier L, Mignard A, Hugnet C, Lefebvre S, ... & Lattard V (2022). Asymptomatic Anticoagulant Rodenticide Exposure in Dogs and Cats—A French and Belgian Rural and Urban Areas Study. Frontiers in Toxicology, 4. doi: 10.3389/ftox.2022.907892
4. Lahmar R, Berny P, Mahjoub T and Ben Youssef S (2019) Animal Pesticide Poisoning in Tunisia. Frontiers in Veterinary Science 6:369. https://doi.org/10.3389/fvets.2019.00369
5. Lahmar R, Mahjoub T, Fourel I et al. (2019) Giant fennel (*Ferula communis L*) intoxication in goats in Tunisia. Vet Record Case Reports, 6(4). doi:10.1136/vetreccr-2018-000738 6



Biography (150-word limit)

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